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Zebrafish Make a Big Splash

Review

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Introduction

The world of developmental biology was changed forever in October 1980 by a paper demonstrating that genes required for embryonic development could be revealed by systematic searches in Drosophila melanogaster (Nüsslein-Volhard and Wieschaus, 1980). Seven months later, a paper suggesting that similar approaches could be taken in Danio (Brachydanio) rerio appeared in the same journal (Streisinger et al., 1981). The article on fly mutations had historic consequences. The article on zebrafish received considerably less attention. However, during the last fifteen years, the world of developmental biology has become increasingly aware of the zebrafish as an experimental organism for understanding vertebrate embryonic development. Today, the genetic potential suggested earlier is about to be realized by the publication in the December 1996 issue of *Development* of 37 papers describing mutations in genes that affect nearly every aspect of zebrafish development (Abdelilah et al., 1996; Baier et al., 1996; Brand et al., 1996a,b; Chen et al., 1996; Driever et al., 1996; Furutani-Seiki et al., 1996; Granato et al., 1996; Haffter et al., 1996; Hammerschmidt et al., 1996a,b; Heisenberg et al., 1996; Jiang et al., 1996; Kane et al., 1996a,b; Karlstrom et al., 1996; Kelsh et al., 1996; Knapik et al., 1996; Malicki et al., 1996a,b; Mullins et al., 1996; Neuhauss et al., 1996; Odenthal et al., 1996a,b; Pack et al., 1996; Piotrowski et al., 1996; Ransom et al., 1996; Schier et al., 1996; Schilling et al., 1996; Solnica-Krezel et al., 1996; Stainier et al., 1996; Stemple et al., 1996; Trowe et al., 1996; van Eeden et al., 1996a,b; Weinstein et al., 1996; Whitfield et al., 1996).

Many of the fundamental principles of vertebrate embryology were elucidated by classical experiments in amphibian and avian embryos early in this century. However, an understanding of the underlying mechanisms has been hampered by the inability to carry out genetic studies in these organisms. The recent, considerable progress in our knowledge of the molecular genetic mechanisms of vertebrate development has relied heavily on two factors: the exciting realization that many developmentally important genes defined by mutations in flies have developmentally important vertebrate counterparts (McGinnis and Krumlauf, 1992; Scott, 1992; De Robertis and Sasai, 1996), and molecular techniques that have provided powerful approaches for identifying genes expressed at specific times and places in vertebrate embryos (Miklos and Rubin, 1996). With the advent of gene targeting in the mouse (Capecchi, 1989), it has become possible to test the function of nearly any cloned gene suspected of participating in the regulation of vertebrate development. While these approaches are enormously powerful, the important lesson from "genetic" organisms such as the fly cannot be ignored. Thus, the most efficient way to find genes that have key regulatory functions in vertebrate development is to screen for them directly by looking for their mutant phenotypes during embryogenesis.

Two groups have now completed the most extensive genetic screens ever carried out in a vertebrate to isolate mutations in genes affecting embryonic development of the zebrafish (Driever et al., 1996; Haffter et al., 1996). This effort has been a tour de force by the laboratories of Christiane Nüsslein-Volhard at the Max Planck Institüt in Tübingen, Federal Republic of Germany, and Wolfgang Driever at Massachusetts General Hospital and Harvard Medical School in Boston, Massachusetts. They were joined in this effort by members of several other laboratories, especially those of Nüsslein-Volhard's colleague Friedrich Bonhoeffer and Driever's colleague Mark Fishman. These screens involved 65 people who examined over a million and a half embryos over about a two year period. Both screens used similar strategies: the F3 progeny of fish carrying chemically-induced point mutations were examined under the stereomicroscope for alterations in a long list of morphological features. Together, these screens resulted in the isolation and initial characterization of an impressive 1858 mutations affecting almost every aspect of embryonic development (Table 1).

Why make zebrafish the focus of such a monumental effort? Zebrafish have a number of features that facilitate recognizing and characterizing mutations (Streisinger et al., 1981). Development is external, making it relatively easy to identify and score mutants either for lethality or for specific and even quite subtle phenotypes. Embryos are optically clear so individual cells can be labeled and their development followed to learn how mutations affect embryonic cell fates (Melby et al., 1996). Individual or groups of cells can be transplanted to new locations to test autonomy of mutant genes (Ho and Kane, 1990). Assays now standard in Xenopus laevis can also be done with zebrafish (Krauss et al., 1992; Hammerschmidt and Nüsslein-Volhard, 1993; Xu et al., 1995; Sagerström et al., 1996) to examine how mutant genes alter embryonic signaling. Finally, genome mapping is now extensive (Postlethwait et al., 1994; Johnson et al., 1995; Knapik et al., 1996) so that it should soon be possible to use positional cloning strategies as well as candidate gene approaches to clone the genes defined by mutations.

This first set of papers provides only a very basic description of most of the mutations. Mutants have typically been categorized by their most prominent phenotypes and it is easy to see from these groupings how mutations affecting genes involved in development of the same embryonic structure could fit together to define developmental pathways. Complementation testing has been done, for the most part, only between mutations within a category and it has not yet been done between mutations isolated in the two separate screens. Since the ability to identify mutations depends on the eyes of the beholder, the distribution of phenotypes isolated by the two screens is somewhat different, and

Table 1. Summary of Screens

	Boston	Tübingen
Number of genomes screened	2337	3857
Number of mutations identified	2383	4264
Number of mutations characterized	695	1163
Number of mutations tested for allelism	331	894
Number of genes defined	220	372
Average allele frequency	1.5	2.4
Number of single allele mutations	164	222

Details of the Boston screen and the Tübingen screen can be found in Driever et al. (1996) and Haffter et al. (1996), respectively.

some mutations which may affect the same process, or represent alleles of the same gene have probably been placed in different categories. Thus, it is difficult to really know the number of genes defined by these mutations. Nonetheless, these papers contain an overwhelming amount of information and this volume will serve as a reference for many years to come. In at least one very real sense, everyone who flips through this issue will gain a new picture of zebrafish development through much of the first embryonic day (Karlstrom and Kane, 1996). In this review, I will focus on a few highlights from the screen papers to illustrate how the new mutations will contribute to our understanding of vertebrate development, and the prospects for the future.

Patterning the Early Embryo

Maternal Effects

Patterning of vertebrate embryos is thought to rely on maternal determinants differentially localized in the egg during its formation (see Weeks and Melton, 1987). Although these screens were designed to identify mutations in zygotically-acting genes, some of the mutations affecting the earliest steps in embryogenesis have maternal effects as well. These were revealed by the finding that, in some cases, heterozygous embryos produced by heterozygous mothers had phenotypes similar to those of homozygous mutant embryos (Hammerschmidt et al., 1996a; Kane et al., 1996a; Mullins et al., 1996). These "zygotic-maternal dominant effects" illustrate overlapping roles for maternal and zygotic genes in establishing the vertebrate body plan. Further searches for mutations in maternally-acting genes will be important to reveal how the complex interplay between maternal and zygotic genes orchestrates embryonic development.

Early Cell Movements

The cellular rearrangments of gastrulation establish the body plan by providing cells with new neighbors and thus opportunities for new interactions. Changes in gastrulation movements alter the body plan. For example, embryos with mutations in the *spadetail* gene, originally described nearly a decade ago (Kimmel et al., 1989) and reisolated in both screens (Hammerschmidt et al., 1996b; Solnica-Krezel et al., 1996) are defective in convergence movements of cells that form paraxial mesoderm, but not those of cells that form axial mesoderm or nervous system (Ho and Kane, 1990). Thus, *spadetail* mutant embryos initally lack somites and have an enlarged tailbud, but the notochord appears relatively normal and alterations in the nervous system appear to be

Figure 1. Embryos with Mutations in the *trilobite* Gene Have Shorter, Broader Somites

Dorsal view, anterior to the left. From Hammerschmidt et al. (1996b), with permission.

secondary effects from loss of somites (Eisen and Pike, 1991). Mutations isolated in new genes in these screens (Hammerschmidt et al., 1996b; Solnica-Krezel et al., 1996), such as *trilobite*, whose name perfectly describes its phenotype (Figure 1), alter gastrulation movements in other regions of the embryo, making embryos with different defects in their body plans. Despite detailed knowledge of the cellular movements of gastrulation, little is known about the underlying molecular genetic mechanisms. For example, it is unclear whether genes defined by mutations such as *spadetail* and *trilobite* control gastrulation movements directly or cause some cells to be misspecified, resulting in incorrect movements (see Kimmel et al., 1991). Detailed characterization of embryos with mutations affecting gastrulation movements, including eventual identification of the genes, should resolve this issue. In addition, understanding the novel cellular juxtapositions in these mutants may reveal heretofore unrecognized cellular interactions that occur during normal embryogenesis.

Establishing the Body Axes

The end result of gastrulation is the emergence of an embryo with clearly established dorsal/ventral (D/V) and anterior/posterior (A/P) body axes. Both screens identified mutations in genes required for establishing D/V cell fates (Hammerschmidt et al., 1996a; Mullins et al., 1996; Schier et al., 1996; Solnica-Krezel et al., 1996). Embryos with mutations in genes such as *swirl* and *snailhouse* have a expansion of dorsal cell fates, such as neurectoderm, at the expense of ventral cell fates, such as tail fins, blood, and posterior somites (Mullins et al., 1996). Some of these mutations have maternal effects as well, making the genes they define candidates for components of a ventralizing pathway that starts functioning during oogenesis and continues to function zygotically. Conversely, mutations in genes such as *dino* and *mercedes* produce embryos with expansion of ventral cell fates at the expense of dorsal fates (Hammerschmidt et al., 1996a). Recent work has implicated BMP4 as the vertebrate ventralizing signal, which appears to function by antagonizing dorsalizing signals that promote neurogenesis (De Robertis and Sasai, 1996). The *dino* and *mercedes* mutant phenotypes are mimicked by overexpression of *bmp4* in wild-type embryos (Hammerschmidt et al., 1996a); thus the genes

defined by these mutations might encode components of the dorsalizing signal, its reception, or downstream effects. BMP4 is a homolog of D. melanogaster Decapentaplegic, a signal involved in dorsalizing the fly embryo by antagonizing the effects of a ventralizing signal, Short gastrulation, whose vertebrate homolog, Chordin, is antagonized by BMP4 (De Robertis and Sasai, 1996). Thus, although insects and vertebrates have body plans that are reversed about the D/V axis, at least some of the molecular mechanisms involved in establishing this body axis are conserved. It will be exciting to use the zebrafish D/V patterning mutations to learn the extent of conservation and to identify differences that may be fundamental for establishing parts of the body plan that are unique to vertebrates.

Do any of the mutations isolated in these screens have phenotypes that suggest a role in A/P patterning? From extensive work in other segmentally organized creatures, especially flies, we have come to expect that A/P patterning involves both broad territories and discrete, iterated regions. *dino* mutants have smaller heads, as might be expected of a mutation in a gene required in a specific A/P territory, but they also have an enlargement of ventrally-derived structures. In vertebrates, patterning of the D/V and A/P axes is interrelated (see Ruiz i Altaba and Melton, 1990). Thus, it is not surprising that although this mutation has been interpreted as affecting D/V patterning (Hammerschmidt et al., 1996a), it also affects patterning along the A/P axis. Mutations in genes affecting cranial neural crest–derived branchial arches alter development of all arches or of restricted groups of adjacent arches (Schilling et al., 1996; Piotrowski et al., 1996; Neuhauss et al., 1996). Since these arches are considered segmentally iterated homologs (Goodrich, 1930), this raises the possibility that specific regions of cranial neural crest are specified together by genes controlling segmental patterning in the head. It will be of great interest to learn whether any of these mutations represent homeotic transformations, as might be expected if they define A/P patterning genes.

Astonishing new expression studies provide clear evidence for repeating patterns that prefigure segmentation along the vertebrate A/P axis (see Kimmel, 1996). Müller et al. (1996) have described the expression pattern of *her1*, a zebrafish homolog of the fly gene *hairy* (Nüsslein-Volhard and Wieschaus, 1980). In flies, *hairy* is expressed in the primordia of alternating parasegments, and *hairy* mutations result in deletion of the regions in which it is normally expressed (Ingham et al., 1985). In zebrafish, *her1* is expressed in the primordia of alternating somites. Although mutations deleting alternating somites were not isolated in these screens, several mutations in genes affecting somite formation (van Eeden et al., 1996a) have phenotypes reminiscent of fly mutations in other segmentally iterated A/P patterning genes. For example, mutations such as *fused somites* and *beamter* have poorly formed boundaries between all somites that could represent transformations or deletions of a portion of each somite. Mutations in *deadly seven* and *after eight* have similar phenotypes, but not in the first five somites. This is intriguing because somite five appears to be the first one expressing *her1*. Clearly, considerably more work will be required to learn whether any of these mutations represent alterations in A/P patterning and, if so, to identify the mechanisms underlying the patterning changes.

Tissue and Organ Formation

Notochord

The notochord arises from axial mesoderm and is central for patterning other parts of the embryo, such as nervous system and somites. Both screens isolated a series of mutations in genes that define stages in notochord development (Odenthal et al., 1996b; Stemple et al., 1996). Mutations in three genes affect specification of notochord precursors. An old friend, *floating head* (Talbot et al., 1995), and a new gene, *bozozok* (Solnica-Krezel et al., 1996; Stemple et al., 1996), affect notochord specification throughout the embryo, while another new gene, *momo* (Odenthal et al., 1996b), only affects notochord specification in the trunk. Mutations in these genes are severe, and many notochord functions appear to be eliminated (for example, patterning of the nervous system). Mutations in several genes affect the transition from precursors to definitive notochord. Some, such as another old friend, *no tail* (Halpern et al., 1993), and two new ones, *gnome* (Stemple et al., 1996) and *doc* (Odenthal et al., 1996b), leave nervous system patterning essentially intact. This is puzzling, since studies in chick suggest that notochord is required to induce ventral nervous system cell types such as floor plate and motoneurons (Yamada et al., 1991). Notochord precursors have been proposed to be present in *no tail* mutant embryos, suggesting that notochord is specified and makes the signaling molecules, such as *sonic hedgehog*, that are important for nervous system patterning, but fails to differentiate. *no tail* mutant embryos do lack some aspects of notochord signaling, since muscle pioneers, a specific subset of identified muscle cells, are absent (Halpern et al., 1993). Recent work (Currie and Ingham, 1996) provides evidence that muscle pioneers are induced by a collaboration between Sonic hedgehog and an additional family member, Echidna hedgehog, which is absent in *no tail* mutant embryos. Thus, notochord specification may be a stepwise process rather than occuring in an all-or-none fashion. This idea can best be tested by learning about the interactions among the genes affecting early notochord development. Moreover, interactions between genes affecting early and late processes of notochord differentiation will help elucidate the developmental pathways required for formation of this important organ.

A particularly interesting set of mutations is the *you* group (van Eeden et al., 1996a). These six mutations were not categorized as affecting notochord, since they have no obvious notochord phenotype. Instead, they affect formation of cell types thought to require notochord signaling, such as nervous system and somites. Thus, this group represents candidates for genes in the notochord-derived signaling pathway. With any luck at all, these mutations could define the signals, their receptors, and downstream events. These mutations should significantly enhance our understanding of the role played by the notochord in organizing adjacent regions of the embryo.

Central Nervous System

Both screens isolated mutations in a large number of genes that have central nervous system (CNS) phenotypes (Abdelilah et al., 1996; Brand et al., 1996a; Furutani-Seiki et al., 1996; Heisenberg et al., 1996; Jiang et al., 1996; Schier et al., 1996). Some of these genes, such as *spiel ohne grenzen* (Schier et al., 1996), *masterblind* (Heisenberg et al., 1996), *no isthmus*, and *acerebellar* (Brand et al., 1996b), affect specific regions of the CNS. The *no isthmus* gene has been identified by linkage analysis and sequencing mutant alleles; the mutations are in the gene formerly called *pax-b*, a zebrafish homolog of the D. melanogaster gene *paired*. Vertebrates have at least nine *paired* homologs, many of which are expressed in specific CNS regions during development. Mutations in three of these *pax* genes have severe developmental defects in mice and humans, suggesting that these genes are important regulators of vertebrate embryogenesis (Mansouri et al. 1994). Two particularly exciting mutations are *white tail* (Jiang et al., 1996) and *mind bomb* (Schier et al., 1996). Mutations in both of these genes have phenotypes that are reminiscent of mutations in fly "neurogenic" genes, in which excess neurons are produced at the expense of epidermis. In both *white tail* and *mind bomb* mutants there is hyperplasia of early-developing neurons and, at least in *white tail* mutants, this is at the expense of later-developing neurons. Mutations in neurogenic genes also affect numerous other cell fate decisions in flies (Parody and Muskavitch, 1993). Consistent with this, both *mind bomb* and *white tail* mutants have defects in pigmentation. Based on their intriguing phenotypes, these mutations may affect genes in the lateral signaling pathway involving Notch and Delta (see Campos-Ortega, 1993). Even if they prove to affect other genes, rather than zebrafish homologs of fly neurogenic genes, further characterization of these mutations should provide important information about the role of cellular interactions in the specification of vertebrate neuronal fates.

Neural Crest and Placodes

The neural crest has been described as "the only interesting thing about vertebrates" (see Thorogood, 1989). Neural crest cells and its associated neurogenic placodes arise at the border between neural and nonneural ectoderm. Placodes give rise to all of the cranial sensory structures and the lens of the eye, as well as the lateral lines of fish and amphibians. Neural crest cells migrate extensively throughout the embryo, giving rise to neurons and support cells of the peripheral nervous system, pigment cells, cranial cartilages, and in fish, to some fin structures (see Eisen and Weston, 1993). Because neural crest and placodes are unique to vertebrates, they may well constitute a place to learn about new genes that function uniquely during vertebrate development, or about the deployment of known genes in new ways.

Pigment cells are a prominent and easily visible neural crest derivative. It is revealing as to the nature of these screens that a very large number of mutations in genes affecting embryonic pigmentation patterns were isolated (285 mutations defining 94 loci; Kelsh et al., 1996; Odenthal et al., 1996a). Because they are easy to recognize, mutations with prominent phenotypes are likely to be over-represented and the genome may have been saturated for them. Zebrafish have three different types of pigment cells. Mutations in only one gene, *colourless* cause loss of all three types, raising the possibility that a single precursor gives rise to all of them (Bagnara et al., 1979). However, lineage data show that pigment cells can arise from clones that also produce other neural crest derivatives (Raible and Eisen, 1994), raising a question as to whether there is a lineal relationship among the different pigment cell types. An exciting possibility is that *colourless* defines a gene important for specification of all trunk neural crest; this possibility can be explored by learning whether other neural crest derivatives, such as neurons or glia, are also affected. Most other pigmentation mutations resulted in the absence of only a single type of pigment cell, although mutations in some genes affected aspects of differentiation of all three types. As with *colourless*, it will be important to learn whether other neural crest derivatives are affected by these mutations. Together, the phenotypes of mutations in genes affecting pigmentation make a nice series defining a variety of processes of neural crest development, including specification, proliferation, patterning, differentiation, and survival. Further study of these mutations will address currently controversial questions about the mechanisms by which neural crest cell fates are specified, as well as questions about how cells are distributed in periodic patterns during vertebrate embryogenesis.

Blood

A fascinating set of mutations are those affecting blood formation (Ransom et al., 1996; Weinstein et al., 1996). Hematopoietic cells are derived from self-renewing, multipotent stem cells that are specified early in embryonic development and continue to generate new blood cells throughout life. Mutations in many genes affecting blood formation are likely to be lethal and thus difficult to study in mammals. Both screens isolated mutants that are bloodless, mutants that appear to affect the ability of hematopoietic stem cells to differentiate into erythroid progenitors, and mutants that have phenotypes resembling human blood diseases. For example, mutants with hypochromic phenotypes, such as *chardonnay*, *chianti*, *grenache*, *sauternes*, *weißherbst*, and *zinfandel*, may represent mutations in genes causing embryonic thalassemias—defects in the globin genes that affect significant percentages of some human populations. Perhaps the most intriguing of these mutations are those with photosensitive blood, such as *freixenet*, *yquem*, *dracula*, and *desmodius* (Figure 2). Humans with congenital erythropoietic porphyrias, defects in heme biosynthesis, have blood that is autofluorescent, as do these four mutants. Characterization of these mutations provides an opportunity to define the molecular components of the hematopoietic system in a way currently unapproachable in other vertebrates and may provide crucial information for understanding diseases of the human hematopoietic system.

Heart and Internal Organs

Organogenesis involves precise coordination, as cells originating in different tissues come together to form a functional unit. Both screens isolated mutations in genes affecting formation of the heart and other internal organs

Figure 2. Embryos with Mutations in the *freixenet* Gene Have Autofluorescent Blood

Side view, anterior to the left. From Ransom et al. (1996), with permission.

(Chen et al., 1996; Pack et al., 1996; Stainier et al., 1996). Interestingly, none of the mutants described were missing an entire organ, although some are missing a region of an organ, such as *lonely atrium* (Chen et al., 1996), which has no ventricle. Mutations in other genes, such as *cloche* (Stainier et al., 1995, 1996), which affects both formation of the heart endocardium and early blood differentiation, will allow testing of models about the relationships between specific cell types during organ formation. Since relatively little is known about the molecular genetics of vertebrate organogenesis, unraveling the steps that orchestrate this vital process should provide new information about regulation of important cellular interactions.

Formation of Neural Connections

Once the fate of neurons has been specified, they must form the enormous diversity of structural and functional characteristics appropriate for their functions. Integral to this is the formation of processes—axons and dendrites—that navigate through the developing embryo and form the intricate networks of interconnections required for proper nervous system function. A long-standing goal of neural development is learning the mechanisms whereby neuronal processes find and recognize their appropriate synaptic partners. One of the most exciting aspects of the studies described here was an additional screen designed to address precisely this issue: How do the processes of retinal ganglion cells find their appropriate targets in the optic tectum? This screen was conducted, in conjunction with the Nüsslein-Volhard screen, in the lab of Friedrich Bonhoeffer (Baier et al., 1996; Trowe et al., 1996; Karlstrom et al., 1996). All of the F2 families used in the original screen were rescreened at a developmental stage when wild-type larvae have an orderly mapping of retinal axons onto their tectal targets. To make this work, a cleverly designed apparatus was used to label axons from two different parts of the retina with two different colors of fluoresent dye. Since these axons normally project to two different regions of the tectum, deviations from their normal projections were readily apparent using fluorescence optics. A large number of mutations affecting genes involved in various aspects of retinal axon targeting were found (Figure 3). Interestingly, most of the mutations identified in this screen were identified by other phenotypes in the Tübingen screen, as well. For example, *bashful*, *sleepy*, and *grumpy* mutants all have

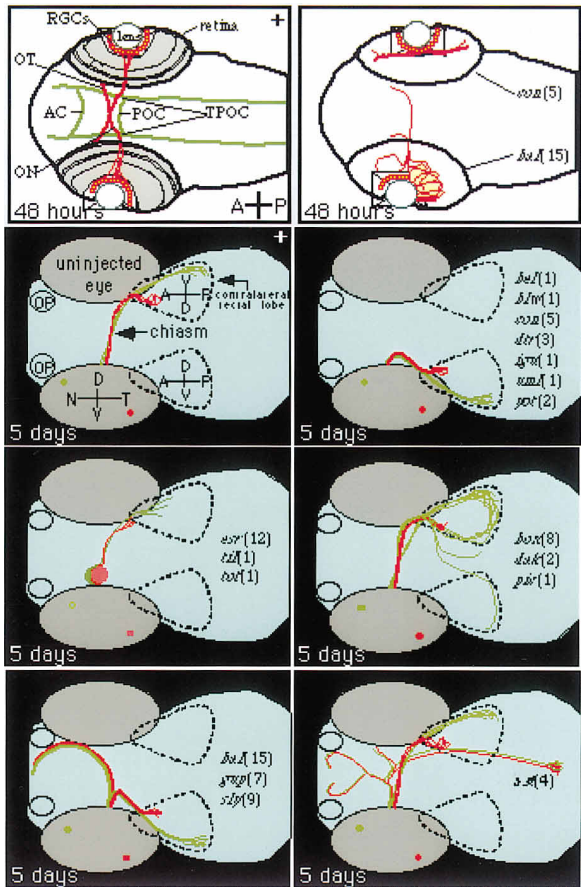

Figure 3. Schematic Overview of Retinotectal Pathfinding Mutants

Figure is from and mutants are described in Karlstrom et al. (1996). Figure is used with permission.

notochord defects (Odenthal et al., 1996b) and *esrom*, *tilsit*, and *tofu* mutants all have xanthophore defects (Odenthal et al., 1996a) as well as defects in retinal axon trajectories. This suggests that many genes may have direct or indirect involvement in numerous developmental processes. Analysis of these mutations will clearly contribute to our understanding of the intrinsic properties of neurons and the environmental signals required for establishing neural networks in the visual system, as well as elsewhere in the animal.

Behavior

Large-scale behavioral screens were first proposed in zebrafish about 15 years ago (Streisinger et al., 1981). Small-scale behavioral screens have already resulted in the identification of a mutant that lacks functional nicotinic acetylcholine receptors (Westerfield et al., 1990) as well as of other mutations affecting genes involved in muscle motility (Felsenfeld et al., 1990). In addition to muscle motility mutants, the Tübingen screen yielded a number of interesting mutants defective in touch responsiveness and rhythmic movements (Granato et al., 1996). Embryos of fish and amphibians normally exhibit alternating contractions of the swimming muscles on opposite sides of the body because

of glycinergic reciprocally inhibitory interneurons that prevent motoneurons on opposite sides from firing at the same time (Dale, 1985). One of the most interesting groups of mutants is missing this inhibition; thus they contract muscles on both sides of the body simultaneously, and move like accordions. Blocking glycineric neurotransmission in wild-type animals phenocopies the mutation, providing evidence that the defect is in the pathway of reciprocal inhibitory signaling. Further analysis of these mutations will enhance our understanding of the neuromuscular signaling pathways involved in establishing early embryonic behaviors, many of which may be important later in life as well (Lee and Eaton, 1991; Bekoff, 1992).

Future Prospects

Given the incredible number of mutations now available affecting genes involved in almost every aspect of zebrafish development, what does the future hold? First and foremost, these mutations represent a treasure trove of tools for studying the mechanisms regulating vertebrate embryogenesis. A person reading these papers may well feel, as I did, like a child in a candy store, picking out a favorite here and another there, and utterly overwhelmed by the possibilities.

Despite the impressive number of new genes defined by mutant phenotypes, it is clear that there are many more yet to be found. This is evident from the large number of mutants discarded because of their "difficult" phenotypes and the large number of mutations represented by only a single allele. Further, mutations with subtle phenotypes could have been easily overlooked, and some mutations in single genes may not yield visible phenotypes because the gene functions are redundant.

What kinds of approaches will be useful for uncovering mutations in additional genes? Work in flies (Seeger et al., 1993) has clearly demonstrated the utility of markers to screen for defects in specific types of neurons. Similar screens using antibodies to recognize neural crest–derived neurons (Henion et al., 1996) and RNA probes to recognize specific CNS regions (Moens et al., 1996) have already provided evidence that this approach can be applied profitably in zebrafish, even in rather small scale screens. Interacting genes should be revealed in carefully designed enhancer/suppressor screens, and conditional mutations (Johnson and Weston, 1995) will be important for revealing when gene functions are required. While all of these screens will be more difficult than examining embryos under the stereomicroscope, they are probably not more difficult than the screen for retinal axon projections. Furthermore, since the potential for finding mutations affecting very specific processes is high, and since the characterization of these mutations will undoubtedly provide new insights into the mechanisms of vertebrate development, the difficulty of the screen is not necessarily a concern.

To really understand the mechanisms of vertebrate development, it will be necessary to characterize the genes that have been revealed by these and other mutations. There is now a map of the zebrafish genome in which each chromosome is represented by a single linkage group (Postlethwait et al., 1994; Johnson et al., 1995), and new markers, including cloned genes, are being added to the map daily. Addition of a sufficient number of cloned genes and recognition of syntenic relationships with other vertebrate species should reveal candidate genes for some of the mutations, once they are mapped. Of course, one of the hopes of an enterprise such as the screens undertaken here is that there will be many new, unknown genes that will provide a new understanding of developmental mechanisms. Positional cloning of these genes will be hard work; however, as in other species (Collins, 1995; Dietrich et al., 1995), there is every indication that this goal will be realized. A zebrafish reference cross anchored to simple sequence length polymorphisms (Knapik et al., 1996) will be especially important for mapping, as a prelude to molecular cloning, because these markers should be informative in most crosses. Furthermore, exciting new work demonstrates the feasibility of highly-efficient generation of insertional mutations in zebrafish (Gaiano et al., 1996), which could circumvent labor-intensive positional cloning.

Despite the obvious advantages of zebrafish for cellular, molecular, and genetic studies of vertebrate development, it is clear that developmental studies of zebrafish will enhance rather than supplant, developmental studies in other vertebrates. For example, the mouse will continue to be the animal of choice for testing the role of cloned genes using embryonic stem cell and homologous recombination techniques not yet established in zebrafish. Avian and amphibian embryos have advantages over zebrafish for some experimental manipulations and as assay systems for testing some signaling molecules. And, of course, although developmental biologists tend to focus on the amazing molecular genetic similarities between animals with disparate body forms, there are clearly important species-specific differences. Investigating the differences will be important to know what things are fundamental and how evolution has acted upon them to produce such a wide variety of vertebrate species. Thus, as we move into the next millenium, the zebrafish will be important for understanding molecular-genetic mechanisms underlying vertebrate development, but a full understanding will only be acquired by complementary studies in a variety of vertebrate species.

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References

Abdelilah, S., Mountcastle-Shah, E., Harvey, M., Solnica-Krezel, L., Schier, A.F., Stemple, D.L., Malicki, J., Neuhauss, S.C.F., Zwartkruis, F., Stainier, D.Y.R., Rangini, Z., and Driever, W. (1996). Mutations affecting neural survival in the zebrafish, *Danio rerio*. Development *123*, 217–227.

Bagnara, J.T., Matsumoto, J., Ferris, W., Frost, S.K., Turner, W.A., Jr., Tchen, T.T., and Taylor, J.D. (1979). Common origin of pigment cells. Science *203*, 410–415.

Baier, H., Klostermann, S., Trowe, T., Karlstrom, R.O., Nüsslein-Volhard, C., and Bonhoeffer, F. (1996). Genetic dissection of the retinotectal projection. Development *123*, 415–425.

Bekoff, A. (1992). Neuroethological approaches to the study of motor development in chicks: achievements and challenges. J. Neurobiol. *23*, 1486–1505.

Brand, M., Heisenberg, C.-P., Warga, R., Pelegri, F., Karlstrom, R.O., Beuchle, D., Picker, A., Jiang, Y.-J., Furutani-Seiki, M., van Eeden, F.J.M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D., Kelsh, R., Mullins, M., Odenthal, J., and Nüsslein-Volhard, C. (1996a). Mutations affecting development of the midline and general body shape during zebrafish embryogenesis. Development *123*, 129–142.

Brand, M., Heisenberg, C.-P., Jiang, Y.-J., Beuchle, D., Lun, K., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D., Kelsh, R., Mullins, M., Odenthal, J., van Eeden, F.J.M., and Nüsslein-Volhard, C. (1996b). Mutations in zebrafish genes affecting the formation of the boundary between midbrain and hindbrain. Development *123*, 179–190.

Campos-Ortega, J.A. (1993). Early neurogenesis in *Drosophila melanogaster*. In The Development of *Drosophila melanogaster*. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press), pp. 1091–1129.

Capecchi, M.R. (1989). Altering the genome by homologous recombination. Science *244*, 1288–1292.

Chen, J.-N., Haffter, P., Odenthal, J., Vogelsang, E., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Kelsh, R.N., Mullins, M.C., and Nüsslein-Volhard, C. (1996). Mutations affecting the cardiovascular system and other internal organs in zebrafish. Development *123*, 293–302.

Collins, F.S (1995). Postional cloning moves from perditional to traditional. Nature Genet. *9*, 347–350.

Currie, P.D., and Ingham, P.W. (1996). Induction of a specific muscle cell type by a hedgehog-like protein in zebrafish. Nature *382*, 452–455.

Dale, N. (1985). Reciprocal inhibitory interneurones in the Xenopus embryo spinal cord. J. Physiol. *363*, 61–70.

De Robertis, E.M., and Sasai, Y. (1996). A common plan for dorsoventral patterning in bilateria. Nature *380*, 37–40.

Dietrich, W.F., Copeland, N.G., Gilbert, D.J., Miller, J.C., Jenkins, N. A., and Lander, E.S. (1995). Mapping the mouse genome: Current status and future prospects. Proc. Natl. Acad. Sci. USA *92*, 10849–10853.

Driever, W., Solnica-Krezel, L., Schier, A.F., Neuhauss, S.C.F., Malicki, J., Stemple, D.L., Stainier, D.Y.R., Zwartkruis, F., Abdelilah, S., Rangini, Z., Belak, J., and Boggs, C. (1996). A genetic screen for mutations affecting embryogenesis in zebrafish. Development *123*, 37–46.

Eisen, J.S., and Pike, S.H. (1991). The *spt-1* mutation alters segmental arrangement and axonal development of identified neurons in the spinal cord of the embryonic zebrafish. Neuron *6*, 767–776.

Eisen, J.S., and Weston, J.A. (1993). Development of the neural crest in the zebrafish. Dev. Biol. *159*, 50–59.

Felsenfeld, A.L., Walker, C., Westerfield, M., Kimmel, C., and Streisinger, G. (1990). Mutations affecting skeletal muscle myofibril structure in the zebrafish. Development *108*, 443–459.

Furutani-Seiki, M., Jiang, Y.-J., Brand, M., Heisenberg, C.-P., Houart, C., Beuchle, D., van Eeden, F.J.M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., and Nüsslein-Volhard, C. (1996). Neural degeneration mutants in the zebrafish, *Danio rerio*. Development *123*, 229–239.

Gaiano, N., Allende, M., Amsterdam, A., Kawakami, K., and Hopkins, N. (1996). Highly efficient germ-line transmission of proviral insertions in zebrafish. Proc. Natl. Acad. Sci. USA *93*, 7777–7782.

Goodrich, E.S. (1930). Studies on the Structure and Development of Vertebrates. (London: Macmillan and Co. Ltd). Reprinted 1986. (Chicago: University of Chicago Press).

Granato, M., van Eeden, F.J.M., Schach, U., Trowe, T., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., and Nüsslein-Volhard, C. (1996). Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva. Development *123*, 399–413.

Haffter, P., Granato, M., Brand, M., Mullins, M.C., Hammerschmidt, M., Kane, D.A., Odenthal, J., van Eeden, F.J. M., Jiang, Y.-J., Heisenberg, C.-P., Kelsh, R.N., Furutani-Seiki, M., Vogelsang, E., Beuchle, D., Schach, U., Fabian, C., and Nüsslein-Volhard, C. (1996). The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio.* Development *123*, 1–36.

Halpern, M.E., Ho, R.K., Walker, C., and Kimmel, C.B. (1993). Induction of muscle pioneers and floor plate is distinguished by the zebrafish *no tail* mutation. Cell *75*, 99–111.

Hammerschmidt, M., and Nüsslein-Volhard, C. (1993). The expression of a zebrafish gene homologous to *Drosophila snail* suggests a conserved function in invertebrate and vertebrate gastrulation. Development *119*, 1107–1118.

Hammerschmidt, M., Pelegri, F., Mullins, M.C., Kane, D.A., van Eeden, F.J.M., Granato, M., Brand, M., Furutani-Seiki, M., Haffter, P., Heisenberg, C.-P., Jiang, Y.-J., Kelsh, R.N., Odenthal, J., Warga, R.M., and Nüsslein-Volhard, C. (1996a). *dino* and *mercedes*, two genes regulating dorsal development in the zebrafish embryo. Development *123*, 95–102.

Hammerschmidt, M., Pelegri, F., Mullins, M.C., Kane, D.A., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Haffter, P., Heisenberg, C.-P., Jiang, Y.-J., Kelsh, R.N., Odenthal, J., Warga, R.M., and Nüsslein-Volhard, C. (1996b). Mutations affecting morphogenesis during gastrulation and tail formation in the zebrafish, *Danio rerio*. Development *123*, 143–151.

Henion, P.D., Raible, D.W., Beattie, C.E., Stoesser, K.L., Weston, J.A., and Eisen, J.S. (1996). Screen for mutations affecting development of zebrafish neural crest. Dev. Gen. *18*, 11–17.

Heisenberg, C.-P., Brand, M., Jiang, Y.-J., Warga, R.M., Beuchle, D., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., and Nüsslein-Volhard, C. (1996). Genes involved in forebrain development in the zebrafish, *Danio rerio*. Development *123*, 191–203.

Ho, R.K., and Kane, D.A. (1990). Cell-autonomous action of zebrafish *spt-1* mutation in specific mesodermal precursors. Nature *348*, 728–730.

Ingham, P.W., Howard, K.R., and Ish-Horowicz, D. (1985). Transcription pattern of the Drosophila segmentation gene *hairy*. Nature *318*, 439–445.

Jiang, Y.-J., Brand, M., Heisenberg, C.-P., Beuchle, D., Furutani-Seiki, M., Kelsh, R.N., Warga, R.M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D.A., Mullins, M.C., Odenthal, J., van Eeden, F.J.M., and Nüsslein-Volhard, C. (1996). Mutations affecting neurogenesis and brain morphology in the zebrafish, *Danio rerio*. Development *123*, 205–216.

Johnson, S.L, Africa, D., Horne, S., and Postlethwait, J.H. (1995). Half-tetrad analysis in zebrafish in zebrafish: mapping the *ros* mutation and the centromere of linkage group I. Genetics *139*, 1727–1735.

Johnson, S.L., and Weston, J.A. (1995). Temperature-sensitive mutations that cause stage-specific defects in zebrafish fin regeneration. Genetics *141*, 1583–1595.

Kane, D.A., Hammerschmidt, M., Mullins, M.C., Maischein, H.-M., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Haffter, P., Heisenberg, C.-P., Jiang, Y.-J., Kelsh, R.N., Odenthal, J., Warga, R.M., and Nüsslein-Volhard, C. (1996a). The zebrafish epiboly mutants. Development *123*, 47–55.

Kane, D.A., Maischein, H.-M., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kelsh, R.N., Mullins, M.C., Odenthal, J., Warga, R.M., and Nüsslein-Volhard, C. (1996b). The zebrafish early arrest mutants. Development *123*, 57–66.

Karlstrom, R.O., and Kane, D.A. (1996). A flipbook of zebrafish embryogenesis. Development *123*, 461.

Karlstrom, R.O., Trowe, T., Klostermann, S., Baier, H., Brand, M.,

Crawford, A.D., Grunewald, B., Haffter, P., Hoffmann, H., Meyer, S.U., Müller, B.K., Richter, S., van Eeden, F.J.M., Nüsslein-Volhard, C., and Bonhoeffer, F. (1996). Zebrafish mutations affecting retinotectal axon pathfinding. Development *123*, 427–438.

Kelsh, R.N., Brand, M., Jiang, Y.-J., Heisenberg, C.-P., Lin, S., Haffter, P., Odenthal, J., Mullins, M.C., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Kane, D.A., Warga, R.M., Beuchle, D., Vogelsang, L., and Nüsslein-Volhard, C. (1996). Zebrafish pigmentation mutations and the processes of neural crest development. Development *123*, 369–389.

Kimmel, C.B. (1996). Was *Urbilateria* segmented? Trends Genet. *12*, 329–376.

Kimmel, C.B., Kane, D.A., and Ho, R.K. (1991). Lineage specification during early embryonic development of the zebrafish. In Cell-Cell Interactions in Early Development. (New York: Wiley-Liss, Inc.), pp. 203–225.

Kimmel, C.B., Kane, D.A., Walker, C., Warga, R.M., and Rothman, M.B. (1989). A mutation that changes cell movement and cell fate in the zebrafish embryo. Nature *337*, 358–362.

Knapik, E.W., Goodman, A., Atkinson, O.S., Roberts, C.T., Shiozawa, M., Sim, C.U., Weksler-Zangen, S., Trolliet, M.R., Futrell, C., Innes, B.A., Koike, G., McLaughlin, M.G., Pierre, L., Simon, J.S., Vilallonga, E., Roy, M., Chiang, P.-W., Fishman, M.C., Driever, W., and Jacob, H.J. (1996). A reference cross DNA panel for zebrafish (*Danio rerio*) anchored with simple sequence length polymorphisms. Development *123*, 451–460.

Krauss, S., Maden, M., Holder, N., and Wilson, S.W. (1992). Zebrafish *pax[b]* is involved in the formation of the midbrain-hindbrain boundary. Nature *360*, 87–89.

Lee, R.K.K., and Eaton, R.D. (1991). Identifiable retinculospinal neurons of the adult zebrafish, *Brachydanio rerio*. J. Comp. Neurol. *304*, 34–52.

Malicki, J., Neuhauss, S.C.F., Schier, A.F., Solnica-Krezel, L., Stemple, D.L., Stainier, D.Y.R., Abdelilah, S., Zwartkruis, F., Rangini, Z., and Driever, W. (1996a). Mutations affecting development of the zebrafish retina. Development *123*, 263–273.

Malicki, J., Schier, A.F., Solnica-Krezel, L., Stemple, D.L., Neuhauss, S.C.F., Stainier, D.Y.R., Abdelilah, S., Rangini, Z., Zwartkruis, F., and Driever, W. (1996b). Mutations affecting development of the zebrafish ear. Development *123*, 275–283.

Mansouri, A., Stoykova, A., and Gruss, P. (1994). Pax genes in development. J. Cell Sci. Suppl. *18*, 35–42.

McGinnis, W., and Krumlauf, R. (1992). Homeobox genes and axial patterning. Cell *68*, 283–302.

Melby, A.E., Warga, R.M., and Kimmel, C.B. (1996). Specification of cell fates at the dorsal margin of the zebrafish gastrula. Development *122*, 2225–2237.

Miklos, G.L.G., and Rubin, G.M. (1996). The role of the genome project in determining gene function: insights from model organisms. Cell *86*, 521–529.

Moens, C.B., Yan, Y.-L., Appel, B., Force, A., and Kimmel, C.B. (1996). *valentino*: a zebrafish gene required for normal hindbrain segmentation. Development, in press.

Müller, M., Weizsäcker, E., and Campos-Ortega, J.A. (1996). Expression domains of a zebrafish homologue of the Drosophila pair-rule gene *hairy* correspond to a primordia of alternating somites. Development *122*, 2071–2078.

Mullins, M.C., Hammerschmidt, M., Kane, D.A., Odenthal, J., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Haffter, P., Heisenberg, C.-P., Jiang, Y.-J., Kelsh, R.N. and Nüsslein-Volhard, C. (1996). Genes establishing dorsoventral pattern formation in the zebrafish embryo: the ventral specifying genes. Development *123*, 81–93.

Neuhauss, S.C. F., Solnica-Krezel, L., Schier, A.F., Zwartkruis, F., Stemple, D.L., Malicki, J., Abdelilah, S., Stainier, D.Y.R., and Driever, W. (1996). Mutations affecting craniofacial development in zebrafish. Development *123*, 357–367.

Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in Drosophila. Nature *287*, 795–801.

Odenthal, J., Rossnagel, K., Haffter, P., Kelsh, R.N., Vogelsang, E., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Mullins, M.C. and Nüsslein-Volhard, C. (1996a). Mutations affecting xanthophore pigmentation in the zebrafish, *Danio rerio*. Development *123*, 391–398.

Odenthal, J., Haffter, P., Vogelsang, E., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Hammershmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Warga, R.M., Allende, M.L., Weinberg, E.S., and Nüsslein-Volhard, C. (1996b). Mutations affecting the formation of the notochord in the zebrafish, *Danio rerio*. Development *123*, 103–115.

Pack, M., Solnica-Krezel, L., Malicki, J., Neuhauss, S.C.F., Schier, A.F., Stemple, D.L., Driever, W., and Fishman, M.C. (1996). Mutations affecting development of zebrafish digestive organs. Development *123*, 321–328.

Parody, T.R., and Muskavitch, M.A.T. (1993). The pleiotrophic function of *Delta* during postembryonic development of *Drosophila melanogaster*. Genetics *135*, 527–539.

Piotrowski, T., Schilling, T.F., Brand, M., Jiang, Y.-J., Heisenberg, C.-P., Beuchle, D., Grandel, H., van Eeden, F.J. M., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Warga, R.M., and Nüsslein-Volhard, C. (1996). Jaw and branchial arch mutants in zebrafish II: anterior arches and cartilage differentiation. Development *123*, 345–356.

Postlethwait, J.H., Johnson, S.L., Midson, C.N., Talbot, W.S., Gates, M., Ballinger, E.W., Africa, D., Andrews, R., Carl, T., Eisen, J.S., Horne, S., Kimmel, C.B., Hutchinson, M., Johnson, M., and Rodriguez, A. (1994). A genetic linkage map for the zebrafish. Science *264*, 699–703.

Raible, D.W., and Eisen, J.S. (1994). Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. Development *120*, 495–503.

Ransom, D.G., Haffter, P., Odenthal, J., Brownlie, A., Vogelsang, E., Kelsh, R.N., Brand, M., van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Mullins, M.C. and Nüsslein-Volhard, C. (1996). Characterization of zebrafish mutants with defects in embryonic hematopoiesis. Development *123*, 311–319.

Ruiz i Altaba, A., and Melton, D.A. (1990). Axial patterning and the establishment of polarity in the frog embryo. Trends Genet. *6*, 57–64.

Sagerström, C.G., Grinblat, Y., and Sive, H. (1996). Anteroposterior patterning in the zebrafish, *Danio rerio*: an explant assay reveals inductive and suppressive cell interactions. Development *122*, 1873–1883.

Schier, A.F., Neuhauss, S.C.F., Harvey, M., Malicki, J., Solnica-Krezel, L., Stainier, D.Y.R., Zwartkruis, F., Abdelilah, S., Stemple, D.L., Rangini, Z., Yang, H., and Driever, W. (1996). Mutations affecting the development of the embryonic zebrafish brain. Development *123*, 165–178.

Schilling, T.F., Piotrowski, T., Grandel, H., Brand, M., Heisenberg, C.-P., Jiang, Y.-J., Beuchle, D., Hammerschmidt, M., Kane, D.A., Mullins, M.C., van Eeden, F.J.M., Kelsh, R.N., Furutani-Seiki, M., Granato, M., Haffter, P., Odenthal, J., Warga, R.M., Trowe, T., and Nüsslein-Volhard, C. (1996). Jaw and branchial arch mutants in zebrafish I: branchial arches. Development *123*, 329–344.

Scott, M.P. (1992). Vertebrate homeobox gene nomenclature. Cell *71*, 551–553.

Solnica-Krezel, L., Stemple, D.L., Mountcastle-Shah, E., Rangini, Z., Neuhauss, S.C.F., Malicki, J., Schier, A.F., Stainier, D.Y.R., Zwartkruis, F., Abdelilah, S., and Driever, W. (1996). Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish. Development *123*, 67–80.

Seeger, M., Tear, G., Ferres-Marco, D., and Goodman, C.S. (1993). Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline. Neuron *10*, 409–426.

Stainier, D.Y.R., Fouquet, B., Chen, J.-N., Warren, K.S., Weinstein, B.M., Meiler, S., Mohideen, M.-A.P.K., Neuhauss, S.C.F., Solnica-Krezel, L., Schier, A.F., Zwartkruis, F., Stemple, D.L., Malicki, J.,

Driever, W., and Fishman, M.C. (1996). Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. Development *123*, 285–292.

Stainier, D.Y., Weinstein, B.M., Detrich, III, H.W., Zon, L.I., and Fishman, M.C. (1995). *Cloche*, an early acting zebrafish gene, is required by both the endothelial and hematopoietic lineages. Development *121*, 3141–3150.

Stemple, D.L., Solnica-Krezel, L., Zwartkruis, F., Neuhauss, S.C.F., Schier, A.F., Malicki, J., Stainier, D.Y.R., Abdelilah, S., Rangini, Z., Mountcastle-Shah, E., and Driever, W. (1996). Mutations affecting development of the notochord in zebrafish. Development *123*, 117–128.

Streisinger, G., Walker, C., Dower, N., Knauber, D., and Singer, F. (1981). Production of clones of homozygous diploid zebrafish (*Brachydanio rerio*). Nature *291*, 293–296.

Talbot, W.S., Trevarrow, B., Halpern, M.E., Melby, A.E., Far, G., Postlethwait, J.H., Jowett, T., Kimmel, C.B., and Kimelman, D. (1995). A homeobox gene essential for zebrafish notochord development. Nature *378*, 150–157.

Thorogood, P. (1989). Developmental and evolutionary aspects of the neural crest. TINS *12*, 38–39.

Trowe, T., Klostermann, S., Baier, H., Granato, M., Crawford, A.D., Grunewald, B., Hoffmann, H., Karlstrom, R.O., Meyer, S.U., Müller, B., Richter, S., Nüsslein-Volhard, C., and Bonhoeffer, F. (1996). Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, *Danio rerio*. Development *123*, 439–450.

van Eeden, F.J.M., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Warga, R.M., Allende, M.L., Weinberg, E.S., and Nüsslein-Volhard, C. (1996a). Mutations affecting somite formation and patterning in the zebrafish, *Danio rerio*. Development *123*, 153–164.

van Eeden, F.J.M., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Warga, R.M., and Nüsslein-Volhard, C. (1996b). Genetic analysis of fin formation in the zebrafish, *Danio rerio*. Development *123*, 255–262.

Weeks, D.L., and Melton, D.A. (1987). A maternal mRNA localized to the vegetal hemisphere in Xenopus eggs codes for a growth factor related to TGF-β. Cell *51*, 861–867.

Weinstein, B.M., Schier, A.F., Abdelilah, S., Malicki, J., Solnica-Krezel, L., Stemple, D.L., Stainier, D.Y.R., Zwartkruis, F., Driever, W., and Fishman, M.C. (1996). Hematopoietic mutations in the zebrafish. Development *123*, 303–309.

Westerfield, M., Liu, D.W., Kimmel, C.B., and Walker, C. (1990). Pathfinding and synapse formation in a zebrafish mutant lacking functional acetylcholine receptors. Neuron *4*, 867–874.

Whitfield, T.T., Granato, M., van Eeden, F.J.M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., and Nüsslein-Volhard, C. (1996). Mutations affecting development of the zebrafish inner ear and lateral line. Development *123*, 241–254.

Xu, Q., Alldus, G., Holder, N., and Wilkinson, D.G. (1995). Expression of truncated *Sek-1* receptor tyrosine kinase disrupts the segmental restriction of gene expression in the Xenopus and zebrafish hindbrain. Development *121*, 4005–4016.

Yamada, T., Placzek, M., Tanaka, H., Dodd, J., and Jessell, T.M. (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. Cell *64*, 635–647.